Comparative Effects on Intestinal Absorption *in Situ* by P-glycoprotein–Modifying HIV Protease Inhibitors

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Purpose. P-glycoprotein (P-gp) is made responsible for the limited oral bioavailability of P-gp substrates like peptidic HIV protease inhibitors (PIs). With respect to combined application of two PIs in antiretroviral regimes, we first investigated the influences on intestinal saquinavir uptake using different PIs in *in situ* perfusion studies.
Methods. Perfusion experiments were carried out in three intestinal segments with P-gp substrates talinolol and saquinavir using fixed concentrations of PIs and with each varying concentrations in the jejunum and ileum. Furthermore, cellular uptake of fluorescent P-gp substrate rhodamine-123 and MRP-substrate carboxyfluorescein has each been quantified in P-gp and MRP-expressing cells by flow cytometry under co-adminstration of PIs.
Results. Increase of calculated permeabilities of P-gp–specific substrate talinolol was found under co-administration of both PIs, ritonavir and H17, with highest absorption rates in the ileal and colon segment. H17 proved to be a better P-gp inhibitor than ritonavir by resulting IC_{50} values and also in the cellular uptake of rhodamine. Similar increases of permeabilities in ileum and colon have also been found for saquinavir as P-gp as well as MRP-substrate with differences in the jejunal uptake, which was found higher for H17. Additional MRP-inhibitory activities of H17 were proved by increasing cellular uptake rates of carboxyfluorescein in MRP-expressing cells.
Conclusions. The investigated PIs were characterized as effective P-gp inhibitors in the intestinal absorption of P-gp substrates. H17 showed MRP-inhibitory effects that also favor intestinal drug absorption of corresponding substrates. With respect to combined therapeutic application of PIs, compounds like H17 raise hopes for improved bioavailability of poorly absorbed compounds.

KEY WORDS: absorption; HIV protease inhibitor; intestine; perfusion; P-glycoprotein.

INTRODUCTION

Peptidic HIV protease inhibitors (PIs) play an important role in the combined antiretroviral therapies against HIV infections, where they maintain a leading position in first-line regimes with disappointing results of novel promising monotherapeutic fusion inhibitors, which turned out as alternatives only for such combined therapies (1,2). One main problem of all the peptidic agents is poor oral bioavailability, which in the case of fusion inhibitor enfuvirtide affords parenteral application (2). The other problem is emerging resistance development which turned out as cross-resistance of almost all peptidic PIs (2).

Membrane efflux pump P-glycoprotein (P-gp) was made responsible for limited oral bioavailability of most PIs, which were characterized as P-gp substrates in *mdr1a* (–/–) (knockout) mice, various P-gp expressing cell models combined with known P-gp inhibitors like cyclosporin A, its analog PSC 833, and verapamil and in comparison of cellular uptake rates in parental and MDR-sublines (3–5). Combinations of PIs in P-gp expressing cell models were reported to be favorable with a resulting increase in celluar uptake suggesting an inhibition function for PI ritonavir as well as not to be of advantage with respect to increasing drug uptake (5,6). Beside promising benefits of therapeutical combinations of poorly absorbed PIs like saquinavir and ritonavir as potential P-gp inhibitor for oral bioavailabilities (7), combination of saquinavir and ritonavir are of current interest to increase the P-gp limited saquinavir uptake across the blood-brain barrier and so reach the brain, as one P-gp caused HIV reservoir as well as across the maternal-fetal barrier to prevent HIV infections of children of HIV positive mothers during delivery (3,5,8). However, clinical investigations of saquinavir suggested poor effects of P-gp inhibition on increased bioavailabilities, which was mainly discussed to result from inhibition of intestinal CYP3A-4 in co-administered therapies. Saquinavir metabolites themselves have been found exclusively in blood with following renal elimination (9).

Inhibitory activties of both P-gp as well as CYP3A-4 each depend on substrate as well as on inhibitor affinities and although some suggestions have been made to correlate those activities final conclusions find this a rather sensitive problem (7). However, recent studies presume increased intestinal metabolism rates for indinavir after P-gp induction by dexamethasone (10).

We decided to characterize the intestinal absorption of poorly absorbed PI saquinavir in competition studies with ritonavir and a novel nonpeptidic PI H17 (2) in *in situ* perfusion studies for realistic estimation of the P-gp role in drug absorption of combined P-gp substrate application. These information may be of worth also to jugde chances of combined PI-regimes for overcoming P-gp mediated cross-resistance caused by lowered uptake rates in human HIV reservoirs like brain. In order to characterize a P-gp specific inhibition of the used compounds P-gp specific model substrate R/S-talinolol has been used in comparison to saquinavir which has been suggested to be an additional substrate of multidrug resistance associated protein (MRP)-types in recent reports (11,12). Results of the studies have been confirmed by *in vitro* experiments in P-gp as well as MRP-expressing cell models.

MATERIALS AND METHODS

Chemicals

Vinblastine was purchased from GRY-Pharma GmbH (Kirchzarten, Germany). R/S-Talinolol and R/S-pindolol were a gift from Arzneimittelwerk Dresden (Radebeul, Germany). Saquinavir was supplied from Novartis-Forschungsinstitut GmbH (Wien, Austria). Ritonavir and its analog A-86093.0 were a gift from Abbott GmbH (Wiesbaden, Germany). H17 was synthesized as previously described (2). Ve-

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rapamil was optained from EGIS Works (Budapest, Hungary). Indomethacin and probenecid were a gift from Berlin-Chemie, (Berlin, Germany) and Biokanol Pharma GmbH (Rastatt, Germany), respectively. Rhodamine 123 and 5-carboxyfluorescein diacetate acetoxymethyl ester (5-CFDA, AM) were purchased from Sigma (St. Louis, MO, USA).

Animals

Experiments were performed in male White Wistar rats purchased from Charles River Laboratories (Sulzfeld, Germany) and were approved by the local ethics committee for animal studies (Regierungspräsidium Dessau/Sachsen-Anhalt). Rats were housed individually in a cage under controlled conditions (21°C, 45% air humidity, 12-h light cycle). They were acclimatized for at least 2 weeks before experiments and had access to tap water and rodent pellet food from Altromin (Lage, Germany).

Intestinal Perfusion Experiments

Perfusion experiments were performed with rats of a weight of 290–350 g that were fasted overnight before studies with access to tap water only. Anesthesia was carried out with Ketamine Inresa (Inresa Arzneimittel GmbH, Freiburg, Germany) (ketamine hydrochloride, 50 mg/kg) and Rompun 2% (xylazin hydrochlorid, 10 mg/kg) (Bayer, Leverkusen, Germany) by intraperitoneal administration. Then rats were placed on a heating pad to maintain body temperature at 37°C. The abdomen was opended by a midline longitudinal incision. For competition studies with fixed substrate/inhibitor concentrations 10 μM talinolol or 20 μM saquinavir and each 30 μM of ritonavir and 20 μM of H17, respectively, and finally 100 μM vinblastine as positive control were used. Three intestinal segments of each rat (n = 3) were selected and perfused over a lenght of each 7–10 cm (jejunum), ~ 5 cm (ileum), and 2 cm (colon) in a three-step procedure with a perfusate flow rate of 0.5 ml/min using an infusion pump from Ismatec (Wertheim-Mondfeld, Germany). After a preceeding 30-min perfusion period for reaching steady state with each substrate and substrate/inhibitor containing Tyrode buffer at a maintaining temperature of 37°C, the outflow samples were taken from each perfusate over 30 min collecting in 5 min intervals so that six samples resulted from each perfusate and intestinal segment. For concentration dependant studies six varying inhibitor concentrations have been used. Jejunum and ileum of each rat (n = 2 per inhibitor concentration) were perfused with each different inhibitors and concentrations. Perfusion procedure followed the above protocol with the preceding and sample taking periods as described.

Bioanalysis

Sample Extraction

Perfusion samples of talinolol as P-gp substrate were spiked with R/S-pindolol as internal standard (i. st.) (0.1 μM) before extraction and then treated with sodium hydroxide (1 M) as described (13). After following extraction with dichlormethane/isopropanol (95/5) (3 ml) on a horizontal shaker, the extracts were washed twice with sodium hydroxide (0.1 M). The centrifuged organic layer was then removed under nitrogen atmosphere and the dry residue was resolved in methanol for HPLC analysis.

Saquinavir samples were spiked with A-86093.0 as methyl analog of ritonavir (20 μM) as i. st. and alkalined with aquous ammonia (0.1 M). Extraction was carried out by shaking with *tert*-butylmethylether (3 ml). After twice washing with the alkaline medium, extracts were centrifuged and evaporated to dryness under nitrogen atmosphere. Dried residues were solved in methanol for analysis.

HPLC Analysis

HPLC separation of talinolol enantiomers in samples before and after perfusion was carried out on a Chiraspher-NT column as stationary phase (5 μm, 25 cm × 0.4 cm) using ethanol/triethylamine (0.05%) as mobile phase as reported (13). Flow rate on separation was optimized with 0.5 ml per min and substrate detection was monitored UV-spectroscopically at 245 nm wavelength. Average retentions were as follows: R-talinolol 15.5 min, S-talinolol 17.8 min, S-pindolol 19.8 min, R-pindolol 21.0 min, while ritonavir and H17 were not extracted. The specificity of the assay was investigated by analyzing blank plasma samples. No interfering peaks with the peaks of the analyst or the i. st. were allowed. Precision and accuracy of this method was measured as inter- and intraassay precision in three separate runs. Six replicates of three different concentrations of talinolol (500, 2000, and 6000 ng/ml) and in the case of the assay for the protease inhibitors saquinavir, H17, and ritonavir (1000, 5000 and 15000 ng/ml) were determined. Analysis of variance (ANOVA) was used to calculate the inter- and intraassay precision. The accuracy was calculated as the average percentage of the nominal concentration. All analyses were within 15% at each concentration.

The lower limit of quantification does not exceed 15%. Samples were determined as six replicates. For the determination of the limit of detection the difference between a spiked sample and a background sample was tested with a paired *t* test. Significance was considered if p was less than 0.05. Quantities that gave a signal-to-noise ratio of three were selected first for determination of the limit of detection. For talinolol enantiomers this value is 2 ng/ml for the limit of detection and 12 ng/ml for the limit of quantification.

Methanolic saquinavir samples were analyzed on a reverse phase chromatography column RP_{18} (5 μm, 15 cm × 0.5 cm) with phosphate buffer (potassium dihydrogen phosphate, disodium hydrogen phosphate) pH = 5.6 at a flow rate of 0.8 ml per minute resulting in retention times of 11.8 min for saquinavir, 12.4 min for ritonavir, and 13.3 min for H17. Detection was carried out UV-spectroscopically at 240 nm wavelenght. All validation procedures were realized as defined and mentioned above. The values for intra- and interassay precision were for all analytes of this method within 15% at each concentration. The lower limit of quantification for saquinavir was 160 ng/ml. The value for the limit of detection was 83 ng/ml.

The fact that metabolites of saquinavir have not been found in the perfusates hardly surprises because they are proved to undergo exclusively renal elimination and under co-administration of P-gp inhibitors like ritonavir intestinal metabolic rates are supposed to be even reduced (9,10).

Water transport was calculated by weight measurement

of each sample before and after perfusion. Steady state existence regarding the analysts and water uptake was examined *via* the trend test.

$$\text{Water transport}\% = 100 \cdot (w_{in} - w_{out})/w_{in}$$

Where w_{in} and w_{out} represent the solution entering and exiting the intestinal segment. The ratio w_{out}/w_{in} was used to perform given corrections concerning water transport.

Cell Culture and Fluorescence Uptake

The L5178Y mouse T lymphoma cell line which was a gift from the NCI (National Cancer Institute of Health, Bethesda, MD, USA) was infected with the PHA *mdr*-1/A retrovirus as described (14). *MDR*-1 expressing cells were selected by culturing the infected cells in 60 ng/ml colchicine. The L5178Y *mdr* cell and the L5178Y parental cell lines were grown in McCoys 5A medium with 10% heat inactivated horse serum, L-glutamine (2 mM), and antibiotics. The purchased breast cancer cell line MDA MB 231 (ATCC: HTB-26) was grown in Leibovitz's L15 medium with 10% fetal bovine serum, L-glutamine (2 mM), and antibiotics. The cultured mouse T lymphoma cells were each adjusted to a concentration of 2×10^6/ml and resuspended in serum free McCoys 5A medium. Then cells were distributed into 0.5 ml aliquots in Eppendorf centrifuge tubes. Test compounds were added from stock solutions (1.0 mg/ml) and the samples were incubated for 10 min at room temperature. Then 10 μl of rhodamin-123 (5.2 μM as final concentration) were added to the samples, and the cells were incubated for further 20 min at 37°C, then washed twice and resuspended in 0.5 ml phosphate-buffered saline (PBS) for analysis. HTB-26 cells were distributed onto 6-well plates with each well containing 2.5×10^5 cells in 5 ml culture medium and grown for 72 h for the experiment. Then the culture medium was changed with 1 ml serum free medium per well and the tested compounds were added from 1.0 mg/ml stock solutions. After incubation for 10 min at room temperature, 10 μl of the carboxyfluorescein were added (2.6 μM as final concentration). After incubation for further 20 min at 37°C, the cells were washed with PBS and detached with trypsin-versen solution (0.25% (w/v) trypsin–0.03% (w/v) EDTA) to harvest them. Then cells were put into Eppendorf centrifuge tubes, washed once with culture medium and PBS, followed by resuspension in 0.5 ml PBS for measurement. Fluorescence of 1×10^4 cells was measured by flow cytometry using a Beckton Dickinson FACScan instrument. Fluorescence activity ratios (FAR) have been calculated from fluorescence uptake relations of treated and untreated control cell lines.

Data Analysis

With the determined mean concentrations of each P-gp substrates in samples before perfusion (C_{in}) and after perfusion (C_{out}) intestinal permeabilities (P_{eff}) have been calculated as previously described using the following equation (13):

$$P_{eff} = Q^* [(C_{in}/C_{out}) - 1]/2\pi rl$$

where Q* represents the flow rate, r is the radius, and l is the length of the perfused intestine segment. From all single values, arithmetical means and standard deviations (SD) have been calculated. Statistical significance of correlations was determined by performing linear regression analyses with tested significant correlation coefficients $p < 0.05$. All studies were performed at least in triplicates. Statistical significance was expressed using an unpaired Student's *t* test (INSTAT, V3.0).

IC_{50} values have been determind using at least six increasing concentrations of each analyst in each perfused segment as mean of two determinations. The calculated standard error of mean (SEM) correlates with the experimental ascertained mean value. All correlations and nonlinear regressions were realized by applying Microcal Origin version 7.0.

RESULTS AND DISCUSSION

R/S Talinolol Studies

We first investigated the influence on drug permeability of R/S-talinolol in competition studies with high concentrations of co-administered PIs. R/S-Talinolol was selected as primary substrate for P-gp inhibition studies because it was favored by recent works as model substrate for such perfusion studies because of its advantageous pharmakokinetic properties of rapid absorption and low metabolic clearance (11). So its reduced bioavailability was attributed to incomplete absorption and demonstrated to result from P-gp transport in the intestine with a sensivity to the degree of P-gp expression. Moreover, R/S-talinolol as suggested P-gp specific substrate may only have some poor affinity to MRP2 as far as evaluated so that it may be of worth for P-gp specific absorption studies in competition with novel potential P-gp inhibitors (11). The used inhibitors have been ritonavir with some suggested P-gp inhibitory activities in P-gp expressing cell models and H17 belonging to a class of novel nonpeptidic class of PIs for which certain P-gp affinities have been found in radioligand binding studies with ^{3}H-verapamil (15).

As can be derived from the mean values of calculated permeabilities for R- and S-talinolol (Table I), some differences have been found in the ileum with a slightly increased absorption rate of the R-diastereomer compared to the S-derivative. Such differences in stereoselectivity of absorption rates have been reported of R/S-talinolol in similar low-dose application to P-gp expressing cells which decreased with increasing talinolol concentrations (13). However, highest absorption rates for both diastereomers have been found in the jejunal segment, which may correlate with lower expression rates of P-gp in this segment compared to the other perfused ones which have recently been reported (16).

Competition studies with both protease inhibitors at fixed concentrations proved a similar increase in permeability for all segments of each individual rats for ritonavir co-administration compared to each talinolol substrate perfused individuals and a consequent increase in permeability from the jejunal to the colon segments for H17 co-administered experiments. Thus, the degree of permeability increase is found larger for H17 in the ileal and colon regions compared to ritonavir even at the comparable lower inhibitor concentration of H17. So, for the talinolol competition a P-gp inhibition can be stated for both used competitors with a more effective inhibition suggested for H17 compared to ritonavir.

Concentration-dependent inhibition studies have been carried out up to saturation of the P-gp inhibition effect,

Table I. Calculated Intestinal Permeabilities (P_{eff}) (cm/s) for R- and S-Talinolol Alone and Each in Co-Administration with P-gp Inhibitors Ritonavir and H17, $p < 0.05$

	Talinolol		Talinolol/Ritonavir		Talinolol/H17	
Perfusate	R	S	R	S	R	S
Jejunum	2.0 ± 0.7	2.4 ± 0.4	3.8 ± 1.5	4.1 ± 0.7		
	3.2 ± 0.7	2.8 ± 0.7			3.8 ± 0.5	3.7 ± 0.3
Ileum	2.3 ± 0.9	1.7 ± 0.6	3.2 ± 1.1	3.1 ± 1.0		
	2.3 ± 0.7	1.9 ± 0.3			3.6 ± 0.9	3.3 ± 0.5
Colon	2.4 ± 0.5	2.3 ± 0.2	4.0 ± 0.6	3.5 ± 0.4		
	2.5 ± 0.4	2.4 ± 0.5			4.5 ± 0.6	4.1 ± 0.4

which was reached for ritonavir at 90 μM and H17 at 40 μM. Resulting permeabilities with mean values of 6.3 to 6.4×10^{-4} cm/s exceed those for vinblastine control at 100 μM lying within the range of 4.5 to 5.5×10^{-4} cm/s as has been reported (13). So, highest absorption rates can be achieved by an H17-application at half of the concentration for ritonavir that is reported to cause certain cytotoxic effects at concentrations > 50 μM, whereas H17 was reported nontoxic in the used concentration range (2,5). The calculated IC_{50} values (Table II) of P-gp inhibition for H17 of about 24 μM for both intestine segments compared to those of ritonavir with highest values of 40 μM underline a superiority of H17 over ritonavir as P-gp inhibitor of P-gp specific drug uptake for R/S-talinolol model compound.

P-gp inhibitory properties of ritonavir and H17 have additionally been characterized in mouse T lymphoma cells (parental cell and *mdr*/1a-expressing subline) in comparison to verapamil as P-gp inhibitor control at 10 μM as a usual standard concentration for *in vitro* studies of effective *mdr*-reversal.

At 10 μM, ritonavir as well as H17 reached higher P-gp inhibitory activities with resulting FARs of 19.12 for ritonavir and 37.71 for H17 compared to 6.60 for verapamil. An extreme superiority of H17 was found at 1 μM with a FAR of already 34.28 and just 1.56 resulting for ritonavir. These *in vitro* data reflect the better P-gp inhibitory properties of H17 compared to ritonavir as have been found in the *in situ* studies.

Saquinavir Studies

So far saquinavir has been characterized in various *in vitro* models as P-gp substrate with hints to additional affinities to MRP1 or 2 (3–5,12). We first characterized the intestinal absorption behavior in perfusion studies with competing PIs in order to analyze possible differing exsorptive transport-effects compared to P-gp specific talinolol which may result from affection of other transporters like MRP.

Calculated permeabilities of saquinavir alone without competitor (Table III) were found almost similar for all intestinal segments compared to R/S-talinolol. In competition with fixed inhibitor concentrations, higher permeabilities for saquinavir have been found in all segments with increasing absorption rates from jejunum to ileum and, finally, colon, which may correlate with increasing P-gp levels in those intestinal segments. Interestingly, H17 leads to higher absorption rates in the jejunum compared to ritonavir which may be plausible with some affinities of H17 to different efflux pumps like MRP which has recently been reported as transporter in rat jejunal tissues (17). As has been found for R/S-talinolol the lower concentration of H17 leads to higher absorption rates compared to ritonavir which may correspond to the above observed more effective P-gp inhibition properties of H17.

In concentration-dependent studies, a saturation of permeability increase is observed for similar inhibitor concentrations as in the talinolol study with 90 μM for ritonavir and 40 μM for H17. However, H17 reaches higher final absorption rates in the jejunal segment with P_{eff} = 8.3 to 9.3 cm/s than ritonavir with P_{eff} = ~ 7.8 cm/s, which may correspond to additional MRP-inhibition. Compared to vinblastine control the increase of saquinavir substrate permeabilities is even higher for both inhibitors than for the talinolol substrate.

Calculated IC_{50} values suggest H17 to be a better P-gp inhibitor in the ileal segment than ritonavir as has been observed for the concentration-dependent talinolol competition. However, highest difference of IC_{50} values for the jejunal

Table II. Calculated IC_{50} Values (μM) for Each Substrate Uptake Determined from Resulting Permeability Rates in Competition Studies with Increasing Inhibitor Concentrations of Ritonavir and H17, Respectively

	Competitor					
	Ritonavir			H17		
	Substrate					
	Talinolol			Talinolol		
	R	S	Saquinavir	R	S	Saquinavir
Jejunum	35.1 ± 0.5	37.3 ± 0.4	52.0 ± 0.5	23.8 ± 0.5	23.2 ± 0.3	26.6 ± 0.1
Ileum	40.0 ± 0.1	39.7 ± 0.2	50.2 ± 0.4	23.7 ± 0.3	23.6 ± 0.3	35.9 ± 0.3

Table III. Calculated Intestinal Permeabilities (P_{eff}) (cm/s) for Saquinavir Alone and Each in Co-Administration with P-gp Inhibitors Ritonavir and H17, $p < 0.05$

Perfusate	Saquinavir	Saquinavir/Ritonavir	Saquinavir/H17
Jejunum	2.4 ± 0.1	3.3 ± 0.1	
	2.5 ± 0.1		3.7 ± 0.4
Ileum	2.2 ± 0.2	3.5 ± 0.2	
	2.4 ± 0.1		3.6 ± 0.1
Colon	2.1 ± 0.1	3.7 ± 0.3	
	2.4 ± 0.5		4.1 ± 0.2

segment may result from additional MRP-inhibiting affinities of H17 compared to ritonavir which shows almost similar values in both, the jejunal and ileal segments. Additional MRP-inhibiting properties of H17 are also supported by the almost identical IC_{50} values for H17 in the talinolol study with talinolol as almost P-gp specific substrate compared to saquinavir with certain MRP-substrate properties as have been suggested by recent studies.

In order to investigate MRP-inhibitory properties of H17 which have been indicated by our *in situ* studies we measured fluorescence uptake of MRP-specific substrate 5-carboxyfluorescein diacetate acetoxymethyl ester in MRP-expressing HTB-26 cell line using indomethacin as MRP-specific inhibitor as control at 28 μM was measured (18). Resulting FAR of 0.9 for indomethacin was reached with H17 at a concentration of just 2 μM. Probenecid as one of the presently best MRP inhibitors was found somewhat superior at the concentration of 2 μM with a FAR of 1.3. So, H17 could be additionally characterized as MRP-inhibitor in these *in vitro* studies of competing MRP inhibitors.

In conclusion, intestinal perfusion studies suggest P-gp inhibitory properties for both investigated PIs. Calculated IC_{50} values document a certain superiority for nonpeptidic H17 compared to ritonavir strengthened by additional suggested inhibitory effects of MRP for saquinavir uptake. Saturation concentrations for both substrates exceed inhibition effects of vinblastine control proving especially H17 as one of the strongest P-gp inhibitors so far as has been investigated in such perfusion studies. As ritonavir disappointed in first clinical investigations as P-gp inhibitor in co-administered PI-studies, our results raise certian hopes for increasing bioavailability of poorly absorbed PIs by co-administration of those novel nonpeptidic PIs like H17 because of their increased inhibitory properties of intestinal transporters.

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REFERENCES

1. X. Wie, J. M. Decker, H. Lui, Z. Zhang, R. B. Arani, J. M. Kilby, M. S. Saag, X. Wu, G. M. Shaw, and J. C. Kappes. Emergence of resistant immunodeficiency virus type 1 in patients receiving fusion inhibitor (T-20) monotherapy. *Antimicrob. Agents Chemother.* **46**:1896–1905 (2002).
2. A. Hilgeroth. Dimeric 4-aryl-1,4-dihydropyridines: development of a third class of nonpeptidic HIV-1 protease inhibitors. *Min. Rev. Med. Chem.* **2**:235–245 (2002).
3. R. B. Kim, M. F. Fromm, C. Wandel, B. Leake, A. J. J. Wood, and D. M. Roden. The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 Protease inhibitors. *J. Clin. Invest.* **101**:289–294 (1998).
4. L. Profite, V. A. Eagling, and D. J. Back. Modulation of P-glycoprotein function in human lymphocytes and Caco-2 cell monolayers by HIV-1 protease inhibitors. *AIDS* **13**:1623–1627 (1999).
5. M. T. Huisman, J. W. Smit, H. R. Wiltshire, R. M. W. Hoetelmans, J. H. Beijnen, and A. H. Schinkel. P-Glycoprotein limits oral availability, brain, and fetal penetration of saquinavir even with high doses of ritonavir. *Mol. Pharmacol.* **59**:806–813 (2001).
6. J. Drewe, H. Gutmann, G. Fricker, M. Török, C. Beglinger, and J. Huwyler. HIV Protease inhibitor ritonavir: a more potent inhibitor of P-glycoprotein than the cyclosporine analog SDZ PSC 833. *Biochem. Pharmacol.* **57**:1147–1152 (1999).
7. C. Leopold. HIV-protease inhibitors - an overview. *Pharm. Uns. Zeit* **30**:234–239 (2001).
8. M. L. Newell. Mechanism and timing of mother-to-child transmission of HIV-1. *AIDS* **12**:831–837 (1998).
9. G. Moyle and B. Gazzard. Current knowledge and future prospects for the use of HIV protease inhibitors. *Drugs* **51**:701–712 (1996).
10. J. H. Lin, M. Chiba, I.-W. Chen, J. A. Nishime, F. A. Deluna, M. Yamazaki, and Y. J. Lin. Effect of dexamethasone on the intestinal first-pass metabolism of indinavir in rats: evidence of cytochrome P-450 A and P-glycoprotein induction. *Drug Metab. Dispos.* **27**:1187–1193 (1999).
11. H. Spahn-Langguth, G. Baktir, A. Radschuweit, A. Okyar, B. Terhaag, P. Ader, A. Hanafy, and P. Langguth. P-glycoprotein transporters and the gastrointestinal tract: Evaluation of the potential in vivo relevance of in vitro data employing talinolol as model compound. *Int. J. Clin. Pharmacol. Ther.* **36**:16–34 (1998).
12. H. Gutmann, G. Fricker, J. Drewe, M. Toeroek, and D. S. Miller. Interactions of HIV protease inhibitors with ATP-dependant drug export proteins. *Mol. Pharmacol.* **56**:383–389 (1999).
13. A. Hanafy, P. Langguth, and H. Spahn-Langguth. Pretreatment with potent P-glycoprotein ligands my increase intestinal secretion in rats. *Eur. J. Pharm. Sci.* **12**:405–415 (2001).
14. M. M. Cornwell, I. Pastan, and M. M. Gottesmann. Certain calcium channel blockers bind specifically to multidrug-resistant human KB carcinoma membrane vesicles and inhibit drug binding to P-glycoprotein. *J. Biol. Chem.* **262**:2166–2170 (1987).
15. A. Hilgeroth, C. Dressler, S. Neuhoff, H. Spahn-Langguth, and P. Langguth. Dimeric 4-aryl-1,4-dihydropyridines as novel HIV-1 protease inhibitors - affinities to intestinal P-glycoprotein. *Pharmazie* **55**:784–785 (2000).
16. R. Yumoto, T. Murakami, Y. Nakamoto, R. Hasegawa, J. Nagai, and M. Takano. Transport of rhodamine 123, a P-glycoprotein substrate, across rat intestine and Caco-2 cell monolayers in the presence of cytochrome P-450 3A-related compounds. *J. Pharmacol. Experim. Therapeut.* **289**:149–155 (1999).
17. S. D. Flanagan, C. L. Cummins, M. Susanto, X. Liu, L. H. Takahashi, and L. Z. Benet. Comparison of furosemide and vinblastine secretion from cell lines overexpressing multidrug resistance protein (P-glycoprotein) and multidrug resistance-associated proteins (MRP1 and MRP2). *Pharmacology* **64**:126–134 (2002).
18. M. P. Draper, R. L. Martell, and S. B. Levy. Indomethacin-mediated reversal of multidrug resistance and drug efflux in human and murine cell lines overexpressing MRP, but not P-glycoprotein. *Br. J. Cancer* **75**:810–815 (1997).